CYP1A2 and CYP2D6 4-Hydroxylate Propranolol and Both Reactions Exhibit Racial Differences

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ABSTRACT

We have previously shown racial differences in propranolol kinetics, with the largest differences appearing to be in its 4-hydroxylation. The purpose of this study was to identify and confirm the cytochrome P450 enzymes (CYP) with propranolol 4-hydroxylase activity, describe their enzyme kinetics, and determine whether there were racial differences in their catalytic activity. Eleven human recombinant, expressed CYPs were screened, but only CYP1A2 and CYP2D6 possessed propranolol 4-hydroxylase activity. Subsequent studies were conducted in human liver microsomes, including correlation, inhibition, enzyme kinetics, and racial comparison studies. Significant correlations were noted between propranolol 4-hydroxylation and ethoxyresorufin-O-deethylation (marker of CYP1A2 activity), with marked improvement in the correlations when CYP2D6-mediated propranolol 4-hydroxylation was inhibited with quinidine. Inhibition studies showed that quinidine inhibited approximately 55% of propranolol 4-hydroxylation and furaphylline (CYP1A2-selective inhibitor) inhibited about 45% of propranolol 4-hydroxylation. Median (range) parameter estimates of (S)-4-hydroxypropranolol [(S)-HOP] formation were a V_{max} value of 307 (165–2397) and 721 (84–1975) pmol/mg of protein/60 min for CYP1A2 and CYP2D6, respectively, and a K_{m} value of 21.2 (8.9–77.5) and 8.5 (5.9–31.9) μM for CYP1A2 and CYP2D6, respectively. CYP1A2- and CYP2D6-mediated propranolol 4-hydroxylation was about 70 and 100% higher (P < .05 for both), respectively, in African-Americans compared with Caucasians. In summary, we found that both CYP1A2 and CYP2D6 catalyze formation of 4-hydroxypropranolol and that both enzymes exhibited racial differences in this reaction. The observed racial differences in drug metabolism may have relevance to drug efficacy, toxicity, or carcinogen activation for CYP1A2 or CYP2D6 substrates.

Clinical research has historically been conducted in Caucasian males, with the assumption that results from this population would be applicable to women and other racial or ethnic groups. However, an increasing number of studies are showing that ethnic or racial groups may differ in their responsiveness to drugs; they may also differ in their handling of drugs (i.e., pharmacokinetics, hepatic drug metabolism) (Johnson, 2000). In two separate studies, we have documented differences between African-Americans and Caucasians in propranolol kinetics (Johnson and Burlew, 1992; Sowinski et al., 1996). In the first study, we showed that after oral propranolol administration at steady state, (S)-propranolol (active enantiomer) and (R)-propranolol oral clearance values were 34 and 53% higher, respectively, in African-Americans compared with Caucasians (Johnson and Burlew, 1992). To investigate further the mechanism for such differences, we performed a second study in which we determined simultaneous i.v. and oral propranolol kinetics among African-Americans and Caucasians phenotyped as extensive metabolizers of dextromethorphan. Consistent with our previous study, (S)- and (R)-propranolol oral clearances were 53 and 76% higher, respectively, in African-Americans than in Caucasians (Sowinski et al., 1996). Systemic clearance was also significantly higher in African-Americans than in Caucasians. In this study, we also determined fractional metabolic clearances for the three major metabolites of propranolol: 4-hydroxypropranolol (HOP), naphthoxylactic acid, and propranolol glucuronide. Fractional metabolic clearances for all three metabolites tended to be higher in African-Americans, although the largest difference was in propranolol 4-hydroxylation, where average fractional metabolic clearances of (S)-HOP and (R)-HOP were 54 and 83% higher, respectively, in African-Americans. The literature clearly suggests the cytochrome P450 (CYP) isozyme CYP2D6 has propranolol 4-hydroxylase activity.

ABBREVIATIONS: HOP, 4-hydroxypropranolol; CYP, cytochrome P450; HLM, human liver microsome; EROD, ethoxyresorufin O-deethylation.
(Ward et al., 1989; Zhou et al., 1990; Masubuchi et al., 1994); thus, we hypothesized that racial differences in CYP2D6 catalytic activity might be responsible for the differences in propranolol kinetics. We therefore studied metoprolol (a model CYP2D6 substrate) kinetics in a group of African-American and Caucasian men who were phenotyped as extensive metabolizers of dextromethorphan (Johnson and Burlow, 1996). Based on the lack of difference between African-Americans and Caucasians in metoprolol kinetics, we concluded there were no racial differences in the catalytic activity of CYP2D6. Thus, we hypothesized that another high-affinity CYP enzyme was responsible for the racial differences in propranolol 4-hydroxylation. The literature suggests that 8 to 50% of propranolol 4-hydroxylation is mediated via an enzyme or enzymes other than CYP2D6 (Distlerath and Guengerich, 1984; Zhou et al., 1990; Marathe et al., 1994; Masubuchi et al., 1994), although the identity of this enzyme was unknown. Thus, the purpose of this study was to identify the other CYP enzyme or enzymes that are responsible for propranolol 4-hydroxylation, determine the relative contribution and enzyme kinetics of the propranolol 4-hydroxylases, and evaluate whether there were racial differences in the catalytic activities of these enzymes.

**Experimental Procedures**

**Materials.** Racemic propranolol and quinidine were obtained from Sigma Chemical Co. (St. Louis, MO). Furafylline was obtained from Research Biochemicals International (Natick, MA). Recombinant expressed microsomal enzymes were obtained from Gentest Corporation (Woburn, MA) or isolated from Gentest cell line cells according to procedures provided by Gentest. Other reagents used in the incubations and subsequent extraction were obtained from Sigma Chemical Co., J.T. Baker (Phillipsburg, NJ), and Fisher Scientific (Pittsburgh, PA). Cell culture reagents were purchased from Gentest, Fisher Scientific, Sigma Chemical Co., and Calbiochem (San Diego, CA). Solvents used in the sample extraction and HPLC mobile phase were of HPLC grade and were purchased from Fisher Scientific, except reagent-grade diethylamine, which was purchased from Sigma Chemical Co., and USP-grade ethanol, which was purchased from AAPER Alcohol and Chemical Company (Shelbyville, KY). The chiral HPLC column was a Chiralcel OD analytical column (250 × 4.6 mm, 10 μm) that was obtained from Chiral Technologies Inc. (Exton, PA).

The HOP used to construct the HPLC standard curves were synthesized in the Department of Pharmaceutical Sciences at the University of Tennessee, Memphis. Briefly, 4-methoxy-1-naphthol purchased from Aldrich Chemical Co. (Milwaukee, WI) was reacted with epichlorohydrin according to the method of Walker and Nelson (1978) to produce an epoxide. Once the epoxide was formed, the synthesis was completed using the method of Oatis et al. (1981). General solvents used in the synthesis were obtained from Fisher Scientific. All other chemicals required were purchased from Aldrich Chemical Co. (Milwaukee, WI). The identity of the final product was confirmed by melting point, NMR, elemental, and mass spectrometric analyses. A stock solution of HOP was made using methanol as the solvent, and the compound was found to be stable in solution for at least 6 months when stored at ~20°C.

**Liver Microsomal Incubations and HOP Assay.** Human livers were obtained from the University of Tennessee (Memphis), the Liver Tissue Procurement and Distribution System (Minneapolis, MN), the Cooperative Human Tissue Network (Birmingham, AL), and In Vitro Technologies Inc. (Baltimore, MD), and their use was approved by the local institutional review board. Livers were obtained through biopsy or within 1 h of "cross-clamp" (in the case of organ donors). Human liver microsomes (HLMs) were prepared as previously described (Meier et al., 1983) and stored at ~80°C until use. Ice-cold NADPH regenerating system (1/10 of final volume, containing 10 U/ml isocitrate dehydrogenase, 50 mM isocitrate, 10 mM sodium NADP, and 50 mM magnesium chloride) was added to a warm incubation mixture of microsomal protein (0.5 mg) and racemic propranolol in 0.1 M potassium phosphate buffer (pH 7.4). The final incubation volume was 0.25 ml. All experiments were performed in duplicate, were carried out at 37°C for 1 h, and then were stopped by the addition of 12.5 μl of 6% perchloric acid. In validation experiments performed before the initiation of these studies, the reactions were validated to be linear for at least 60 min. Sodium metabisulfate (20 mg) was added to prevent oxidation of HOP. Samples were then extracted and analyzed for (R)- and (S)-HOP by chiral HPLC as described previously (Herring and Johnson, 1993). Briefly, the compounds of interest were extracted into ethyl acetate from alkalized incubation mixtures, concentrated by drying, reconstituted in mobile phase, and injected. The chromatographic separation was effected at room temperature on a Chiralcel OD column (J.T. Baker, Inc.) using a mobile phase of hexane:ethanol:diethylamine:water (90:10:0.1:0.05, v/v/v/v) flowing at a rate of 1 ml/min. The eluting compounds were detected by fluorescence, with excitation at 240 nm and a 320-nm high-pass filter. Retention times were about 9, 12, 20, and 24 min for (R)-propranolol, (S)-propranolol, (S)-HOP, and (R)-HOP, respectively. Standard curves were constructed with the same sample matrix (rat liver microsomes) along with perchloric acid and known concentrations of racemic HOP.

**Identification of CYPs Responsible for Propranolol 4-Hydroxylase.** Recombinant, expressed human microsomal CYPs 1A1, 1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, 2C19, 3A4, and 4A11 were screened for propranolol 4-hydroxylase activity, using the incubation conditions described above, with the exception that incubations were carried out for 2 h, as recommended by the supplier of the recombinant human CYPs. Experiments were also performed in our laboratory that validated linearity of the reaction for 2 h. In the case of some of the recombinant human CYPs, two different alleles of an enzyme (i.e., CYP2D6 and CYP2C9) are available. For these enzymes, we tested the more common allele. Many of the CYP cDNAs have been coexpressed with human cytochrome P450 oxidoreductase, which increases substrate turnover about 3-fold. To determine the degree of metabolism that occurs independent of the CYP of interest, catalytic activity of microsomes from control cell lines or control microsomes expressing oxidoreductase were also determined. Finally, all microsomes (those from cells expressing specific human CYPs and control microsomes) also were studied in the presence and absence of an NADPH regenerating system to confirm the absence of non-CYP metabolism or chemical degradation.

**Confirmation of Propranolol 4-Hydroxylase Activity of CYP1A2.** Correlation and inhibition studies were conducted to confirm positive findings of propranolol 4-hydroxylase activity from the recombinant CYP studies. Correlation between propranolol 4-hydroxylation and ethoxyresorufin O-deethylation (EROD) (model CYP1A2-mediated reaction) was determined for 36 HLM samples. Propranolol 4-hydroxylation was determined with a substrate concentration of 100 μM propranolol in the absence and presence of 2.5 μM quinidine (potent, selective inhibitor of CYP2D6; Nielsen et al., 1990). In preliminary studies, 2.5 μM quinidine was shown to be the lowest concentration that produced maximal inhibition of CYP2D6. Lack of inhibition of CYP1A2 by this concentration of quinidine was previously described (Meier et al., 1983). For the inhibition studies, we determined HOP formation velocity with 100 μM racemic propranolol in the absence of any inhibitor, the presence of 2.5 μM quinidine, and the presence of 100 μM furafylline (potent, selective inhibitor of CYP1A2; Halpert et al., 1994). As described for quinidine, 100 μM furafylline was selected as the lowest concentration that maximally inhibited CYP1A2 and was...
confirmed to lack inhibitory effects on CYP2D6 in human recombinant CYP2D6 microsomes.

**Characterization of CYP1A2 and CYP2D6 Propranolol 4-Hydroxylation Kinetics.** Full enzyme kinetic studies were conducted on six different HLMs, with racemic propranolol concentrations ranging from 1 to 1000 μM. Enzyme kinetics were determined in the absence of any inhibitor, the presence of 2.5 μM quinidine, and the presence of 100 μM furaphylline. Model parameters derived from experiments conducted in the presence of quinidine were assumed to represent the enzyme kinetics for CYP1A2, and parameters derived from experiments conducted in the presence of furaphylline were assumed to represent the enzyme kinetics for CYP2D6.

**Determination of Racial Differences in Propranolol 4-Hydroxylation.** HLMs from African-American donors were identified from the liver bank at St. Jude Children’s Research Hospital. Caucasian donors were then matched for age, race, gender, and smoking status. When possible, two Caucasian donor HLMs were matched to the African-American donor HLM. Matched-pair experiments were conducted on the same day. Formation velocity of HOP was determined using 100 μM racemic propranolol as substrate. (This concentration of propranolol was chosen to reflect maximum hydroxylation; see Fig. 2.) Experiments were conducted in the absence of inhibitor, in the presence of 2.5 μM quinidine, and in the presence of 100 μM furaphylline. Formation velocity of HOP in the presence of quinidine was assumed to represent CYP1A2-mediated propranolol 4-hydroxylation and that in the presence of furaphylline was presumed to represent CYP2D6-mediated propranolol 4-hydroxylation.

**Data Analysis.** Correlations between formation velocities of propranolol 4-hydroxylation and ethoxyresorufin-O-deethylation were determined by Spearman rank correlation. Percentage of inhibition of the formation velocity for HOP by quinidine and furaphylline was calculated as an estimate of the contribution of CYP2D6 and CYP1A2 to propranolol 4-hydroxylation. Data from enzyme kinetic experiments were fitted to the Michaelis-Menten equation using a one-site model, a two-site model, or a two-site model with V_max and K_m values of the second site estimated as a ratio. Nonlinear modeling was conducted using WinNonlin (SCI Software, Cary, NC). The model of best fit was determined by visual inspection of the data, evaluation of the correlation matrix for the parameter estimates, evaluation of coefficients of variation of the parameter estimates, and Akaike Information Criterion. Median parameter estimates were used to simulate formation velocity-versus-substrate concentration curves. Comparisons of data from African-American and Caucasian liver donors were made by Mann-Whitney U test.

**Results**

Eleven of the most common drug-metabolizing CYPs were screened for their ability to catalyze the 4-hydroxylation of propranolol (CYPs 1A1, 1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, 2C19, 3A4, and 4A11). Only CYP2D6 and CYP1A2 were found to produce HOP; thus, all subsequent studies focused on these two enzymes. Control microsomes, control microsomes expressing oxidoreductase, and microsomes incubated in the absence of NADPH did not produce HOP.

Correlation studies were conducted to confirm the catalytic activity of CYP1A2 for propranolol 4-hydroxylation. Because CYP2D6 is known to be a major enzyme responsible for propranolol 4-hydroxylation, correlation analyses were conducted in a standard fashion (in the absence of inhibitor) but also in the presence of quinidine to abolish the contribution of CYP2D6. As shown in Fig. 1, the correlation between propranolol hydroxylation and EROD was statistically significant in both the absence and the presence of quinidine; however, the correlation coefficient was much higher when metabolism by CYP2D6 was inhibited with quinidine. Data for (S)-HOP and (R)-HOP were similar and were all highly statistically significant (all P values < .0005). Correlation coefficients were as follows: (S)-HOP: no inhibitor, r = 0.55; with quinidine, r = 0.78; (R)-HOP: no inhibitor, r = 0.56; with quinidine, r = 0.79.

Inhibition studies were conducted in HLMs from 37 donors to confirm catalytic activity for HOP formation and secondarily to assess the relative contributions of CYP2D6 and CYP1A2 to propranolol 4-hydroxylation. (S)-HOP and (R)-HOP formations were inhibited 42 ± 16 and 47 ± 15%, respectively, by furaphylline. Percentage of inhibition by furaphylline ranged from 11 to 82% for (S)-HOP formation and from 16 to 88% for (R)-HOP formation. Quinidine inhibited formation of (S)-HOP and (R)-HOP by 55 ± 17 and 58 ± 17%, respectively. Ranges for percentage of inhibition by quinidine were 11 to 78% for (S)-HOP and 11 to 83% for (R)-HOP.

Enzyme kinetics for HLMs from 6 donors are summarized in Table 1. Median V_max was estimated to be about 2-fold greater for CYP2D6 than for CYP1A2 for the formation of both (S)-HOP and (R)-HOP. K_m values for (S)-HOP formation were about 2-fold higher for CYP1A2 than for CYP2D6, whereas K_m values for (R)-HOP were approximately 10-fold
TABLE 1

Propranolol 4-hydroxylation (HOP) kinetics

<table>
<thead>
<tr>
<th></th>
<th>Total HOP</th>
<th>HOP via CYP1A2</th>
<th>HOP via CYP2D6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_{\text{max}} ) (pmol/mg/h)</td>
<td>( K_{\text{m}} ) (μM)</td>
<td>( V_{\text{max}} ) (pmol/mg/h)</td>
</tr>
<tr>
<td>L-HOP</td>
<td>1010 (496–3208)</td>
<td>24.5 (7.5–69.0)</td>
<td>1237 (555–4362)</td>
</tr>
<tr>
<td></td>
<td>307 (165–2397)</td>
<td>21.2 (8.9–77.5)</td>
<td>395 (303–3579)</td>
</tr>
<tr>
<td></td>
<td>721 (84–1975)</td>
<td>8.5 (5.9–31.9)</td>
<td>701 (83–2873)</td>
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<tr>
<td></td>
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</tbody>
</table>

\( a \) “HOP via CYP1A2” and “HOP via CYP2D6” indicate enzyme kinetics determined in the presence of quinidine and furaphylline, respectively.

\( b \) \( n = 5 \) because it was impossible to obtain reasonable parameter estimates with one HLM when studies were conducted in the presence of quinidine.

\( c \) \( V_{\text{max}} \) units given as picomoles per milligram of protein per 60 minutes.

A higher for CYP1A2. Figure 2 depicts the simulated formation velocity-versus-substrate concentration curves for both (S)-HOP and (R)-HOP, highlighting the differences in enzyme kinetics for CYP1A2 versus CYP2D6.

Liver samples from 16 African-American donors were included in this study. Five of the African-American donors were matched by demographic characteristics with two Caucasian donors each; the remaining 11 African-American donors were matched with one Caucasian donor each. The African-American/Caucasian-matched groups are referred to subsequently as matched sets.) Thus, there were a total of 21 Caucasians included in the analysis. Seven of the matched sets were female \( (n = 16 \) donors), and seven sets were male \( (n = 17 \) donors). The remaining two matched sets were mixed in gender \( (n = 4 \) donors), but all were from donors less than 1 year old, an age at which gender would be less likely to affect drug metabolism. Liver tissue was normal or normal adjacent in 14 of the 16 matched sets and cirrhotic in the remaining two matched sets. African-American and Caucasian donors did not differ by age, with mean ± S.D. ages of 32 ± 5 years for Caucasian donors and 36 ± 25 years for African-American donors. On average, the Caucasian donors were 1.5 years younger than the African-American donor in their matched set.

Racial differences in propranolol 4-hydroxylation are shown in Table 2 and depicted in Fig. 3. Propranolol 4-hydroxylation was significantly higher in African-Americans than in Caucasians via both CYP1A2 and CYP2D6. (S)-HOP and (R)-HOP formation via CYP1A2 was 68 and 94% higher, respectively, in African-Americans than in Caucasians. Similarly, (S)-HOP and (R)-HOP formation via CYP2D6 was 75 and 98% higher, respectively, in African-Americans than in Caucasians. The degree of inhibition by quinidine and furaphylline suggested no racial differences in the relative contribution of CYP1A2 and CYP2D6 to propranolol 4-hydroxylation. For example, furaphylline inhibited (S)-HOP formation by 43 ± 15% in African-Americans and by 40 ± 17% in Caucasians. Similarly, quinidine inhibited (S)-HOP formation by 55 ± 17% in African-Americans and by 54 ± 17% in Caucasians. Because of concern about how ontogeny of CYP1A2 and CYP2D6 activity in infants may have affected our findings, we also performed our analysis after exclusion of data from our four donors who were under the age of one. This analysis revealed more highly significant differences between African-Americans and Caucasians than when the infants were included in the analysis (data not shown).

### Discussion

We have previously shown that propranolol kinetics were significantly different between African-Americans and Caucasians (Johnson and Burlew, 1992; Sowinski et al., 1996), with the formation of HOP exhibiting the greatest racial differences in maximal propranolol 4-hydroxylation rates (pmol/mg/h)

<table>
<thead>
<tr>
<th></th>
<th>African-Americans ( (n = 16) )</th>
<th>Caucasians ( (n = 21) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-HOP</td>
<td>1181 (32–2806)</td>
<td>545 (48–2054)*</td>
</tr>
<tr>
<td>d-HOP</td>
<td>1725 (41–4179)</td>
<td>791 (68–3026)*</td>
</tr>
<tr>
<td>Via CYP1A2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-HOP</td>
<td>481 (13–1357)</td>
<td>286 (16–512)*</td>
</tr>
<tr>
<td>d-HOP</td>
<td>655 (15–1867)</td>
<td>337 (18–167)*</td>
</tr>
<tr>
<td>Via CYP2D6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-HOP</td>
<td>639 (25–1712)</td>
<td>365 (29–1251)*</td>
</tr>
<tr>
<td>d-HOP</td>
<td>862 (21–4246)</td>
<td>435 (41–1695)*</td>
</tr>
</tbody>
</table>

\( a \) Data generated from HLMs from African-American and Caucasian donors.

\( b \) Formation velocities determined in the presence of quinidine.

\( c \) Formation velocities determined in the presence of furaphylline.

\( * P < .05, \) African-Americans versus Caucasians by Mann-Whitney U test.
versus Caucasians by Mann-Whitney U test. (A) Median formation velocities of HOP in HLMs from African-American (■) and Caucasian (□) donors. *P < .05, African-Americans versus Caucasians.

Our data provide compelling evidence that along with CYP2D6, CYP1A2 has propranolol hydroxylase activity that is relevant at therapeutic propranolol concentrations. Specifically, we found that recombinant, expressed human CYP1A2 microsomes had clear-cut propranolol 4-hydroxylase activity. Propranolol 4-hydroxylation was also significantly correlated with ethoxyresorufin-O-deethylation, a marker for CYP1A2-mediated metabolism, and the correlation was markedly improved when CYP2D6-mediated propranolol 4-hydroxylation was inhibited by quinidine. We also found that the selective CYP1A2 inhibitor furaphylline produced significant inhibition of propranolol 4-hydroxylation.

Several lines of evidence also provide information about the relative contribution of CYP1A2 and CYP2D6 to propranolol 4-hydroxylation. The inhibition studies revealed the degree of inhibition by maximally inhibitory concentrations of furaphylline (about 45%) was only slightly less than the degree of inhibition by maximally inhibitory concentrations of the CYP2D6 inhibitor quinidine (about 55%). These data suggest CYP1A2 may be only slightly less important than CYP2D6 in catalyzing this reaction. Conversely, the enzyme kinetic data, which revealed higher $V_{\text{max}}$ and lower $K_m$ values for CYP2D6, suggest that this is the predominant propranolol 4-hydroxylase, especially when one considers that the concentrations accomplished in vivo may be at or below the $K_m$. Thus, precise estimates of the relative contributions of CYP1A2 and CYP2D6 to propranolol 4-hydroxylation cannot be made from our data, particularly with respect to the in vivo pharmacokinetics. However, these data do suggest that CYP1A2 is an important propranolol 4-hydroxylase, and it would almost certainly be the predominant propranolol 4-hydroxylase in the 2 to 10% of the population who are CYP2D6 poor metabolizers. Indeed, it is well documented that the frequency of the CYP2D6 poor metabolizer phenotype is significantly lower in African-Americans than in Caucasians (Relling et al., 1991; Evans et al., 1993), resulting in a larger proportion of Caucasians for whom CYP1A2 would be the predominant propranolol 4-hydroxylase.

Previous studies by Masubuchi et al. (1994) and Yoshimoto et al. (1995) attempted to determine the CYPs responsible for propranolol metabolism. Although their data were somewhat suggestive that CYP1A2 may play a role, its role was less prominent than we observed in our study. A possible explanation for this is that these studies were conducted in HLMs from Japanese donors. Although we did not find any differences between African-Americans and Caucasians in the relative contribution of CYP1A2 to propranolol 4-hydroxylation, it is possible that this isozyme contributes little to propranolol 4-hydroxylation in the Japanese. Ching et al. (1996) investigated propranolol 4-hydroxylation in recombinant, expressed human CYP1A2 and, similar to our findings, showed CYP1A2 to have propranolol hydroxylase activity.

Once we identified CYP1A2 and CYP2D6 as the propranolol 4-hydroxylases, we could proceed with the major objective of our study: to determine whether these enzymes exhibited racial differences in their ability to catalyze formation of HOP. We found that HOP formation velocity was significantly higher in African-Americans than in Caucasians for both enzymes. Specifically, formation velocity of (S)-HOP by both enzymes was about 70% higher in African-Americans, and (R)-HOP formation velocity by both enzymes was about 2-fold higher in African-Americans.

Given that CYP1A2 is highly susceptible to induction by cigarette smoking (Conney; 1986), it is possible that our finding of racial differences in CYP1A2 propranolol 4-hydroxylation is merely a reflection of racial differences in smoking. However, we do not believe this to be the case for several reasons. First, we knew the smoking status of 68% of our donors, and 51% of the donors were nonsmokers. African-Americans and Caucasians were also matched for smoking status whenever possible, and our findings of racial differences among those in whom smoking status was known and matched were similar to those of the whole population. Thus, we think it unlikely that racial differences in smoking status explain the observed racial differences in CYP1A2 catalytic activity. CYP2D6 does not appear to be induced by smoking (Schaaf et al.; 1987); thus, even if there were differences in smoking status between our African-American and Caucasian donors, this would not explain the observed racial differences in CYP2D6-mediated propranolol 4-hydroxylation.

The major purpose of our study was to identify CYPs that might exhibit racial differences in their catalytic activity; it was thought that this would provide information that might be useful in predicting or identifying other xenobiotics (especially drugs and carcinogens) that might also exhibit racial differences in their metabolism, resulting in racial differ-
ences in drug efficacy, toxicity, or cancer risk. Our data clearly suggest both CYP1A2 and CYP2D6 exhibit racial differences in their catalytic activity for propranolol 4-hydroxylation. Whether this translates into racial differences in catalytic activity for numerous other substrates for these enzymes is unclear.

There is little literature evaluating the presence or lack of racial differences in kinetics/metabolism for CYP1A2 substrates. Probably the best studied of the CYP1A2 substrates (with respect to pharmacokinetics) is theophylline, and a population modeling study of theophylline in 84 patients found that African-American race was associated with a 34% higher theophylline clearance than the reference population (Driscoll et al., 1989). Given that hepatic metabolism is responsible for nearly all of the clearance of theophylline and that CYP1A2 is the major isoenzyme responsible for its metabolism (Ha et al., 1995), these data support our finding of higher CYP1A2 catalytic activity in African-Americans than in Caucasians.

Although the theophylline data in African-Americans appear consistent with our finding of greater metabolism via CYP1A2, this is in contrast to previous findings of Relling et al. (1992). In this study, urinary molar concentration ratios of specific caffeine metabolites were used to assess racial differences in CYP1A2 catalytic activity. Based on metabolic ratio data, it was concluded that CYP1A2 catalytic activity was lower in African-Americans than in Caucasians. However, the ratio used as an assessment of CYP1A2 may have also been affected by CYP2A6 (Gu et al., 1992).

CYP1A2 not only is responsible for the metabolism of certain drugs but also causes metabolic activation of certain carcinogens, including aromatic and heterocyclic amines, nitroaromatic compounds, and mycotoxins (Landi et al., 1999). Consistent with this, data suggest that patients with rapid CYP1A2 activity are at an increased risk of colon and bladder cancer (Landi et al., 1999). Thus, higher CYP1A2 activity in African-Americans might contribute to the higher colon cancer incidence in African-Americans (Troisi et al., 1999) and the excess bladder cancer risk from smoking in African-Americans (Burns and Swanson, 1991). Further research is needed to determine whether there are racial differences in metabolism for most CYP1A2 substrates, because such knowledge may provide important insights into racial differences in drug pharmacokinetics and cancer risk.

CYP2D6 is responsible for the metabolism of a large number of commonly used drugs; thus, racial differences in its catalytic activity would be of particular clinical significance. In this study, we found that propranolol 4-hydroxylation via CYP2D6 was 70 to 100% higher in African-Americans. The question is how this finding relates to other CYP2D6 substrates. In a previous study from our laboratory, we studied the pharmacokinetics of metoprolol in healthy male African-American and Caucasian volunteers who were all phenotyped to be extensive metabolizers of dextromethorphan (Johnson and Burlew, 1996). Metoprolol was chosen as a model substrate because approximately 70 to 80% of its metabolism occurs via CYP2D6 (Lennard et al., 1982; Otton et al., 1988). Quinidine is a potent and selective inhibitor of CYP2D6 (Nielsen et al., 1990; Halpert et al., 1994); thus, we determined metoprolol kinetics in the presence and absence of quinidine; the oral clearance of metoprolol that was inhibited by quinidine was defined as metabolism via CYP2D6. In that study, we found metoprolol oral clearances were nearly identical in African-American and Caucasian subjects, in both the presence and the absence of quinidine. Thus, we concluded that there were no racial differences in the metabolism of metoprolol via CYP2D6, at least among extensive metabolizers.

Other data suggest CYP2D6-mediated metabolism may be lower in African-Americans than in Caucasians. For example, a study in 65 depressed patients found that nortriptyline trough plasma concentrations were about 50% higher in African-Americans than in Caucasians (Ziegler and Biggs, 1977). Nortriptyline is a known CYP2D6 substrate, so this finding might suggest lower CYP2D6-mediated metabolism in African-Americans. A number of studies in which probe drugs (most commonly debrisoquine) are used to phenotype patients also suggest lower CYP2D6-mediated metabolism in African-Americans compared with Caucasians (Masimirembwa et al., 1996; Leathart et al., 1998; Wennerholm et al., 1999).

The apparent discrepancy of the effect of race on CYP2D6-mediated drug metabolism appears to be consistent with the literature, suggesting a dissociation of metabolic correlations for CYP2D6 probe drugs among African populations. For example, among Nigerians, Lennard et al. (1992) found a weak correlation between metabolic ratios for metoprolol and sparteine and no correlation between metabolic ratios for metoprolol and debrisoquine, or sparteine and debrisoquine. In contrast, metabolic ratios for these CYP2D6 probe drugs have been shown to be highly correlated among Caucasians (Alvan et al., 1990). Another study of African villagers from Vendaland similarly found no relationship between those identified as poor metabolizers using metoprolol as the probe drug versus using debrisoquine as the probe drug (Sommers et al., 1989). Two separate studies of Zimbabweans and Zambians also found dissociation between the findings with debrisoquine versus metoprolol (Simooya et al., 1993; Masimirembwa et al., 1996).

Based on the literature described above, it appears that there is discordance among various CYP2D6 substrates in African-Americans that is less evident among Caucasians. Specifically, one can make arguments from the literature for higher, lower, and comparable metabolism of various CYP2D6 substrates in African-Americans compared with Caucasians. Thus, there may be little value in the approach of using a single CYP2D6 substrate as a probe drug to provide insight into CYP2D6-mediated metabolism in African-Americans. The basis for the variability in CYP2D6-mediated metabolism in African-Americans is unknown, but it is possible that the different allelic variants of CYP2D6, or racial differences in the expression of “compensatory” enzymes between Caucasians and African-Americans, result in variable interactions with different CYP2D6 substrates. We did not genotype our HLM samples for CYP2D6, although such information may have provided us with additional insight into our observation of greater CYP2D6-mediated 4-hydroxylation among African-Americans.

In summary, we identified that both CYP1A2 and CYP2D6 have propranolol 4-hydroxylase activity and that the formation rate of HOP by both CYP1A2 and CYP2D6 was significantly higher in HLMs from African-American than from Caucasian donors. The observed racial differences in drug
metabolism may have relevance to drug efficacy, toxicity, or carcinogen activation for CYP1A2 or CYP2D6 substrates.

References


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